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(54) Title: INHIBITORS OF INTEGRIN RECEPTORS AND THEIR THERAPEUTICAL USES

(57) Abstract

The present invention concerns inhibitors of activation of at least one integrin receptor for use in promoting the healing of wounds or fibrotic disorders with reduced scarring. Also provided are methods for promoting the healing of wounds or fibrotic disorders with reduced scarring, comprising inhibiting the activation of at least one integrin receptor.

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INHIBITORS OF INTEGRIN RECEPTORS AND THEIR THERAPEUTICAL USES

The present invention concerns inhibitors of at least one integrin receptor for use in promoting the healing of wounds or fibrotic disorders, in particular for promoting the healing of wounds or fibrotic disorders with reduced scarring, and for use in promoting the healing of chronic wounds.

By "wounds or fibrotic disorders" is meant any condition which may result in the formation of scar tissue. In particular, this includes the healing of skin wounds, the repair of tendon damage, the healing of crush injuries, the healing of wounds to the eye, including wounds to the cornea, the healing of central nervous system (CNS) injuries, conditions which result in the formation of scar tissue in the CNS, scar tissue formation resulting from strokes, and tissue adhesion, for example, as a result of injury or surgery (this may apply to e.g. tendon healing and abdominal strictures and adhesions). Examples of fibrotic disorders include pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, systemic sclerosis, scleroderma and proliferative vitreoretinopathy.

In particular, there is a lack of compositions for promoting the healing of wounds or fibrotic disorders with reduced scarring. Scar tissue formation, although providing mechanical strength to a healed wound, can be unsightly and may impair the function of the tissue.

This is particularly the case in wounds which result in scar tissue formation in the CNS, the scar tissue inhibiting the reconnection of severed or re-growing nerve ends, so significantly affecting their function.

There is also a lack of compositions for use in the treatment of chronic wounds, for example venous ulcers, diabetic ulcers and bed sores (decubitus ulcers),

especially in the elderly and wheel chair bound patients. Such compositions may be extremely useful in patients where wound healing is either slow or in whom the wound healing process has not yet started. Such compositions may be used to "kick-start" wound healing and may then be used in combination with compositions (e.g. those of PCT/GB92/00570 and PCT/GB93/00586) which promote the healing of wounds or fibrotic disorders with reduced scarring. Hence not only may a chronic wound be healed, but it may be healed with reduced scarring.

According to the present invention there is provided an inhibitor of activation of at least one integrin receptor for use in promoting the healing of wounds or fibrotic disorders with reduced scarring

Integrins perform various roles at a site of wounding or fibrotic disorder (a "site"). They are involved in the binding of growth factors at the site to e.g. platelets embedded in fibrin clots and are also used by fibroblasts to migrate to the site of the wound. Integrins affect both fibrotic and non-fibrotic growth factors and so it would be supposed that by affecting integrins in general there would not be a beneficial effect upon healing.

The profile of growth factors at a site during the healing process varies over time. Initially, fibrotic growth factors predominate, in paricular fibrotic TGF-β_1 as it is released upon platelet degranulation at initial wounding.

The various members of the TGF-β family are present at the site in various forms - free-TGF-β (which is in its active form), the TGF-β-LAP (TGF-β-latency associated peptide) complex, which is endocytosed by cells (*via* the M-6-P receptor) and which also binds *via* the RGD site in the LAP to the integrin receptor gpIIb/IIIa on the active platelet surface, and the TGF-β-LAP-LTBP (TGF-β-LAP-latent TGF-β binding protein) complex which masks the RGD-peptide binding site on the LAP and so

circulates in the serum but also binds to extracellular matrix molecules. This mechanism provides for a slow release of TGF-β_1 from the fibrin clot to promote wound healing.

Over time, the profile of TGF-β changes in favour of the non-fibrotic TGF-β_3 as fibroblasts migrate into the provisional wound matrix using integrin receptors which bind to ECM (extracellular matrix) molecules and release TGF-β_3.

By inhibiting the activation of integin receptors, the present inventor has found that, surprisingly, inhibitors of integrin receptor activation may be used to promote healing with reduced scarring.

The inhibitor may bind to at least one receptor but not activate it.

The inhibitor may comprise an antibody. It may comprise a neutralising antibody. The antibody may bind specifically to at least one integrin receptor. It may bind specifically to the RGD recognition peptide or an analogue thereof.

The inhibitor may comprise at least the RGD peptide or an analogue thereof.

The inhibitor may be any form of inhibitor which inhibits the activation of at least one integrin receptor. It may, for example, be a neutralising antibody specific to the RGD recognition site of integrins, it may be a neutralising antibody specific to the integrin receptor, or it may contain the RGD peptide or an analogue (e.g. a RGDS peptide or a mimotope of RGD - see for example Geysen, H.M. *et al.*, 1987, Journal of Immunological Methods, 102: 259-274) thereof which will bind to the integrin receptor and prevent the natural ligand from binding to it.

A receptor may be the GpIIb/IIIa platelet receptor. Hence an inhibitor may be any form of a GpIIb/IIIa platelet receptor inhibitor including existing pharmaceutical compounds. The inhibitor may also comprise an RGD peptide or an analogue thereof. Hence a double effect may be achieved by the present invention using a Gp IIb/IIIa inhibitor containing an RGD peptide or an analogue thereof which binds to integrin receptors (e.g. on platelets) and prevents RGD-containing LAP-TGF-β_1 complexes from binding to them, and also prevents the platelet release reaction, thereby reducing the quantity of fibrotic TGF-β_1 at the wound site.

The inhibitor may inhibit the binding of TGF-β_1 and/or platelets or leucocytes to fibrin and/or fibrinogen and/or fibronectin. It may for example be a fibrinogen receptor antagonist.

The inhibitor of activation of at least one integrin receptor may be used in a quantity sufficient to inhibit, or to substantially inhibit, the activation of the integrin receptor, i.e. an activity inhibiting amount of the inhibitor may be used.

The inhibitor of activation of at least one integrin receptor may be used in conjunction with an inhibitor of platelet activation and/or degranulation.

The inhibitor of activation of at least one integrin receptor may be used in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

The inhibitor of activation of at least one integrin receptor may be used in conjunction with a composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

The inhibitor of activation of at least one integrin receptor may be used in conjunction with a composition for promoting the healing of chronic wounds.

Also provided according to the present invention is a method for promoting the healing of wounds and fibrotic disorders comprising inhibiting the activation of at least one integrin receptor.

The inhibition may be achieved by administering to a site an inhibitor of the activation of at least one integrin receptor. The inhibitor may be an inhibitor of activation of at least one integrin receptor according to the present invention.

The integrin may be inhibited immediately prior to wounding/onset (by "onset" is meant the onset of a fibrotic disorder). It may be inhibited immediately after wounding/onset, although it may also be inhibited later, for example within 12, 24, 48, 72, 96 or 120 hours of wounding/onset.

The efficacy of the present invention is significantly enhanced by the inhibition of integrins either immediately before or just after wounding/onset. As described above, the profile of TGF-β at the wound site changes over time, initially favouring fibrotic TGF-βs upon platelet degranulation and the release of TGF-β_1, and later favouring non-fibrotic TGF-β as fibroblasts migrate to the site and release TGF-β_3. By inhibiting integrins primarily at the time when they are being used by predominantly fibrotic TGF-β-LAP to bind to platelets in the fibrin clots, the subsequent release of fibrotic TGF-β may be significantly reduced and hence even more reduced scarring may be achieved.

The method may be used in conjunction with a method for promoting the healing of wounds or fibrotic disorders with reduced scarring.

The method may be used in conjunction with a method for promoting the healing of chronic wounds.

The invention will be further apparent from the following examples which show, by way of example only, forms of promotion of healing of wounds or fibrotic disorders with reduced scarring.

Example 1

An activity-inhibiting amount of neutralising anti-RGD antibody is applied to a site of wounding immediately prior to an incisional wound being made.

Example 2

An acitivity-inhibiting amount of an RGD peptide is applied to a site of wounding immediately after wounding has occurred.

Example 3

An activity-inhibiting amount of neutralising antibody specific to the GpIIa/IIIb platelet receptor is applied to a site of wounding immediately before and after wounding has occurred.

Example 4

An acivity-inhibiting amount of an anti-RGD antibody is applied to a site of fibrosis.

CLAIMS

1. An inhibitor of activation of at least one integrin receptor for use in promoting the healing of wounds or fibrotic disorders with reduced scarring.

2. An inhibitor of activation of at least one integrin receptor according to claim 1 wherein it binds to at least one receptor but does not activate it.

3. An inhibitor of activation of at least one integrin receptor according to either one of claims 1 or 2, comprising an antibody.

4. An inhibitor of activation of at least one integrin receptor according to claim 3, comprising a neutralising antibody.

5. An inhibitor of activation of at least one integrin receptor according to either one of claims 3 or 4 wherein the antibody binds specifically to at least one integrin receptor.

6. An inhibitor of activation of at least one integrin receptor according to either one of claims 3 or 4 wherein it binds specifically to the RGD peptide or an analogue thereof.

7. An inhibitor of activation of at least one integrin receptor according to either one of claims 1 or 2 wherein it comprises at least the RGD peptide or an analogue thereof.

8. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims wherein a receptor is the GpIIb/IIIa platelet receptor.

9. An inhibitor of activation of at least one integrin receptor according to claim 8 wherein it comprises a GpIIb/IIIa platelet receptor inhibitor.

10. An inhibitor of activation of at least one integrin receptor according to claim 9 wherein the GpIIb/IIIa platelet receptor inhibitor also comprises an RGD peptide or an analogue thereof.

11. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims wherein a receptor is the fibrinogen receptor.

12. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims wherein it inhibits the binding of TGF-β_1 and/or platelets or leucocytes to fibrin and/or fibrinogen and/or fibronectin.

13. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims wherein it is an inhibitor of platelet activation and/or degranulation.

14. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims for use in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

15. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims for use in conjunction with a composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

16. An inhibitor of activation of at least one integrin receptor according to any one of the preceding claims for use in conjunction with a composition for promoting the healing of chronic wounds.

17. A method for promoting the healing of wounds or fibroic disorders with reduced scarring comprising inhibiting the activation of at least one integrin receptor.

18. A method according to claim 17 comprising administering to a site an inhibitor of the activation of at least one integrin receptor.

19. A method according to claim 18 wherein it comprises administering an inhibitor of activation of at least one integrin receptor according to any one of claims 1-15.

20. A method according to any one of claims 17-19 wherein the activation of the integrin receptor is inhibited either immediately prior to or immediately after onset.

21. A method according to any one of claims 17-20 for use in conjunction with a method for promoting the healing of wounds or fibrotic disorders with reduced scarring.

22. A method according to any one of claims 17-21 for use in conjunction with a method or composition for promoting the healing of chronic wounds.

INTERNATIONAL SEARCH REPORT

Interr ·nal Application No
PCT/GB 96/02366

A. CLASSIFICATI N OF SUBJECT MATTER
IPC 6 A61K39/395 A61K38/06 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 220 957 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 May 1987 see page 5, line 65 - page 6, line 3 see claims ---	1,2, 7-14, 17-20
X	WO 93 19783 A (THE WHITTIER INSTITUTE FOR DIABETES AND ENDOCRINOLOGY) 14 October 1993 see the whole document ---	1-4,7, 12-14, 17-20
X	WO 93 00108 A (CORVAS INTERNATIONAL, INC.) 7 January 1993 see the whole document --- -/--	1,2, 7-14, 17-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
7 February 1997	26.02.97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 89 11538 A (CENTOCOR ET AL.) 30 November 1989 see the whole document	1-5,8,9, 11-14
X	EP 0 368 486 A (MERCK & CO ET AL.) 16 May 1990 see figures 3,5,6 see examples 4,6	1,2,7-14
X	INVESTIGATIVE OPHTALMOLOGY & VISUAL SCIENCE, vol. 35, no. 5, April 1994, ST. LOUIS, MO, USA, pages 2585-2591, XP000616130 R. HERSHKOVITZ ET AL.: "Nonpeptidic analogogues of the Arg-Gly-Asp (RGD) sequence specifically inhibit the adhesion of human tenon's capsule fibroblasts to fibronectin." see abstract see results	1,7, 17-22
X	DATABASE WPI Week 9323 Derwent Publications Ltd., London, GB; AN 93-184811 XP002024815 & JP 05 111 390 A (NIPPON SHINYAKU CO. LTD.) , 7 May 1993 see abstract	1-3,6
A	WO 92 08982 A (THE SCRIPPS RESEARCH INSTITUTE) 29 May 1992 see examples see claims	1-22
A	WO 92 08739 A (THE SCRIPPS RESEARCH INSTITUTE) 29 May 1992 see examples see claims	1-22

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 96/ 02366

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 17-22 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

formation on patent family members

International Application No
PCT/GB 96/02366

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EP-A-220957	06-05-87	US-A-	4683291	28-07-87
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